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CHAPTER II

Preliminary Classification:
Proposed Class:
Subclass:

TRANSMITTAL LETTER
TO THE UNITED STATES ELECTED OFFICE (EO/US)
(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

EP00/06088	29 June 2000 (29.06.00)	29 June 1999 (29.06.99)
International Application Number	**International Filing Date**	**International Earliest Priority Date**

TITLE OF INVENTION: NEAR FIELD OPTICAL EXAMINATION DEVICE

APPLICANT(S): MUELLER, Gerhard; HELFMANN, Juergen

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

1. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. Section 371:

CERTIFICATION UNDER 37 C.F.R. SECTION 1.10*

(Express Mail label number is ***mandatory.****)*
(Express Mail certification is optional.)

I hereby certify that this paper, along with any document referred to, is being deposited with the United States Postal Service on this date 21 DEC 2001 in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EL 829634085US__, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

_____Stephen L. Grant ___
(type or print name of person mailing paper)

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a. This express request to immediately begin national examination procedures (35 U.S.C. Section 371(f)).

b. The U.S. National Fee (35 U.S.C. Section 371(c)(1)) and other fees (37 C.F.R. Section 1.492) as indicated below:

2. Fees

CLAIMS FEE*	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALC-ULATIONS
	TOTAL CLAIMS	77 -20 =	57	x $18.00 =	$1,026.00
	INDEPEN-DENT CLAIMS	3 - 3 =	0	x $84.00 =	$0.00
	MULTIPLE DEPENDENT CLAIM(S) (if applicable) + $280.00				$0.00
BASIC FEE	U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in Section 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in Section 1.445(a)(2) to the U.S. PTO: where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. Section 1.492(a)(5)) $890.00				$890.00
	Total of above Calculations				= $1,916.00
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed. (note 37 CFR Sections 1.9, 1.27, 1.28)				- $0.00
	Subtotal				$1,916.00
	Total National Fee				$1,916.00
	Fee for recording the enclosed assignment document $40.00 (37 C.F.R. Section 1.21(h)). See attached "ASSIGNMENT COVER SHEET".				$0.00
TOTAL	Total Fees enclosed				$1,916.00

*See attached Preliminary Amendment Reducing the Number of Claims.

A check in the amount of $1,916.00 to cover the above fees is enclosed.

3. A copy of the International application as filed (35 U.S.C. Section 371(c)(2)) has been transmitted by the International Bureau.

Date of mailing of the application (from form PCT/IB/308): 4 January 2001

4. A verified translation of the International application into the English language (35 U.S.C. Section 371(c)(2)) is transmitted herewith.

5. Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. Section 371(c)(3)) have not been transmitted. Applicant chose not to make amendments under PCT Article 19.

Date of mailing of Search Report (from form PCT/ISA/210): 9 September 2000.

6. A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. Section 371(c)(3)) has not been transmitted for reasons indicated in section 5.

7. A copy of the international examination report (PCT/IPEA/409) is transmitted herewith.

8. There is no annex to the international preliminary examination report to transmit herewith.

9. There is no annex to the international preliminary examination report to translate and transmit herewith.

10. An unsigned oath or declaration of the inventor (35 U.S.C. Section 371(c)(4)) complying with 35 U.S.C. Section 115 is submitted herewith, and such oath or declaration identifies the application and any amendments under PCT Article 19 that were transmitted as stated in Section 3 and/or 5; and states that they were reviewed by the inventor as required by 37 C.F.R. Section 1.70.

II. Other document(s) or information included:

11. An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a) is transmitted herewith.

12. An Information Disclosure Statement under 37 C.F.R. Sections 1.97 and 1.98 will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. Section 371(c).

13. Additional documents:

a. Copy of request (PCT/RO/101)

b. International Publication No. 01/01183

Front page only

14. The above items are being transmitted before 30 months from any claimed priority date.

Date: 21 DEC. 2001

Signature of Practitioner

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Attorney's Docket 7040-52

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Helfman, et al. Examiner:

Ser. No.: Art Group:

Title: NEAR-FIELD OPTICAL INVESTIGATION DEVICE

Filed: 21 December 2001 Date: 21 December 2001

PRELIMINARY AMENDMENT

This Preliminary Amendment is filed as a part of the national stage entry of PCT application EP00/06088, which was filed on 29 June 2000, which is in turn based on German application 199 29 875.0, filed on 29 June 1999. The fees for the claims should be calculated based on the claims remaining after the entry of this Preliminary Amendment, which results in 77 total and 3 independent claims. Consistent with the modifications to 37 CFR §1.125, the applicant has provided a substitute specification instead of a clean copy of the paragraphs and claims as they stand after amendment.

Amendments to the Disclosure

The specification as filed has been altered from the literal translation document received to delete information above the title, to insert headings according to US practice, and to insert paragraph numbering in lieu of line numbering. These changes do not introduce new matter.

In addition, please make the following change to the specification:

Amendments to the Claims

After the heading "CLAIMS" and before the beginning of the claims, please insert the words: -- What is claimed is: --

Please amend the claims as follows:

1. (amended) A sample holder [(20; 20'; 34; 36; 38)], in particular for a biological sample [(24)], for use in an apparatus [(10; 10')] for near-field optical imaging, comprising :

a carrier [(20.1; 34.1; 36.1; 38.1, 38.2)] and

a converter [(20.2; 34.2, 34.3; 36.3, 36.3; 38.3)] which is connected to the carrier and which contains a converter material and emits [(34.3, 36.3) and is adapted to emit] light of small lateral source extent upon irradiation with an electron beam [(13)], characterized by an adhesion layer [(20.4; 34.5; 36.5; 38.5)] which fixes [is arranged and designed to fix] the sample [(24)] in the near field of the converter [(20.2; 34.2, 34.3; 36.3, 36.3; 38.3)].

2. (amended) The [A] sample holder of claim 1, wherein [as set forth in claim 1 characterized in that] the adhesion layer has [(20.4; 34.5; 36.5; 38.5) is of] a thickness of a maximum of 30 nm.

3. (amended) The [A] sample holder of claim 1, wherein [as set forth in claim 1 or claim 2 characterized in that] the adhesion layer thickness [of the adhesion layer (20.4; 34.5; 36.5; 38.5)] is constant over a portion of its lengthwise and transverse extent.

4. (amended) The [A] sample holder of claim 1, wherein [as set forth in one of claims 1 through 3 characterized in that] the adhesion layer comprises [(20.4; 34.5; 36.5; 38.5) includes] a lipid or a cellulose derivative.

5. (amended) The [A] sample holder of claim 1, wherein [as set forth in one of claims 1 through 4 characterized in that] the adhesion layer [(20.4; 34.5; 36.5; 38.5)] is [in the form of] a molecule mono-layer or molecule bi-layer.

6. (amended) The [A] sample holder of claim 4, wherein [as set forth in claim 4 or claim 5 characterized in that] the adhesion layer comprises [(20.4; 34.5; 36.5; 38.5) includes] a phospholipid.

7. (amended) The [A] sample holder of claim 4, wherein [as set forth in claim 4, claim 5 or claim 6 characterized in that] the adhesion layer [(20.4; 34.5; 36.5; 38.5)] contains a carboxylmetoxy cellulose.

8. (amended) The [A] sample holder of claim 1, wherein the [as set forth in one of the preceding claims characterized by a] converter material [(34.3; 36.3) which upon electron irradiation] emits light of a spectral width of more than 50 nm wavelength upon electron irradiation.

9. (amended) <u>The</u> [A] sample holder <u>of claim 1, wherein</u> [as set forth in one of the preceding claims characterized in that] the converter material [(34.3; 36.3)] is embedded in small volumes [(34.2; 34.3)] in the carrier.

10. (amended) <u>The</u> [A] sample holder <u>of claim 9, wherein</u> [as set forth in claim 9 characterized in that] the <u>small</u> volumes [(34.2; 36.2)] are regularly repetitively arranged at a small relative spacing in two directions.

11. (amended) <u>The</u> [A] sample holder <u>of claim 1, wherein</u> [as set forth in one of the preceding claims characterized in that] the converter material <u>comprises</u> [(36.3) includes a] phosphorus.

12. (amended) <u>The</u> [A] sample holder <u>of claim 1, wherein</u> [in one of claims 1 through 11 characterized in that] the converter material <u>comprises</u> [(36.3) includes] anthracene.

13. (amended) <u>The</u> [A] sample holder <u>of claim 1, wherein</u> [as set forth in one of the preceding claims characterized in that] the converter material <u>comprises</u> [(34.3) includes] transformed material of the carrier [(34.1)] in small volumes [(34.2)].

14. (amended) <u>The</u> [A] sample holder <u>of claim 1, wherein</u> [as set forth in one of the preceding claims characterized in that] the converter material [(34.3)] contains micro- or nano-porous silicon.

15. (amended) <u>The</u> [A] sample holder <u>of claim 1, wherein</u> [as set forth in one of claims 1 through 8 and 11 through 14 characterized in that] the converter material [(20.2; 38.3)] is homogenously distributed on the carrier [(20.1; 38.1)].

16. (amended) <u>The</u> [A] sample holder <u>of claim 14, wherein</u> [as set forth in claim 14 characterized in that] the carrier forms the converter.

17. (amended) <u>The</u> [A] sample holder <u>of claim 1, wherein</u> [as set forth in one of the preceding claims characterized in that] the carrier [(20.1; 34.1; 36.1; 38.1, 38.2)] comprises a semiconductor material[, preferably Si, SiO_2, Si_3N_4 or GaAs].

18. (amended) The [A] sample holder of claim 1, wherein [as set forth in one of the preceding claims characterized in that] the carrier [(38.1)] is reinforced at spacings of between 10 and 100 μm by an additional thickening [(38.2)].

19. (amended) The [A] sample holder of claim 1, further comprising [as set forth in one of the preceding claims characterized by] a window [(32)] which is transmissive for electrons, on the side of the carrier [(20.1)] which is remote from the adhesion layer [(20.4), the window preferably containing beryllium or silicon].

20. (amended) The [A] sample holder of claim 19, wherein [as set forth in claim 19 characterized in that] the window reinforces [(32) is designed and arranged to reinforce] the carrier [(20.1)].

21. (amended) The [A] sample holder of claim 1, further comprising [as set forth in one of the preceding claims characterized by] a protective layer for protecting the converter from contamination by the sample, [(20.3; 34.4; 36.4; 38.4)] between the converter [(20.2; 34.2, 34.3; 36.3, 36.3; 38.3)] and the adhesion layer [(20.4; 34.5; 36.5; 38.5) which is adapted to protect the converter from contamination by the sample (24)].

22. (amended) The [A] sample holder of claim 1, further comprising [as set forth in one of the preceding claims characterized by] a nutrient solution container [(34)] which is releasably fixed to the sample holder and which receives [is adapted to receive] the sample [(24)] and a nutrient solution.

23. (amended) An apparatus [Apparatus (10; 10')] for near-field optical imaging comprising :

a [an electron beam] source for emitting [(12) adapted to emit] an electron beam ; [(13)] and

a sample holder, comprising: [characterized in that the sample holder (20; 20'; 34; 36; 38) is designed in accordance with one of claims 1 through 21]

a carrier and

a converter that is connected to the carrier and that contains a converter material that emits light of small lateral source extent upon irradiation with the electron beam, characterized by an adhesion layer that fixes the sample in the near field of the converter.

24. (amended) The apparatus of claim 23, further comprising: [Apparatus as set forth in claim 23 characterized by]

a vacuum arrangement such that the [(18) which is designed in such a way that a] converter [(20.2; 34.2, 34.3; 36.3, 36.3; 38.3) connected to the sample holder (20; 20'; 34; 36; 38)] can be excited in vacuum by the electron beam [(13)].

25. (amended) The apparatus of claim 24, wherein [Apparatus as set forth in one of claims 23 and 24 characterized in that the electron beam (13) can be passed by means of] a deflection unit [(14)] passes the electron beam in a scanning raster mode over a [the] plane of the sample holder [(20; 20'; 34; 36; 38)] in which the [a] converter material [(20.2; 34.3; 36.3; 38.3)] is arranged.

26. (amended) The apparatus of claim 25, wherein [Apparatus as set forth in one of claims 23 through 25 characterized in that] the vacuum arrangement [(18)] is such that a sample [(24)] to be investigated is disposed on a [the] side of the converter [(20)] remote from the electron beam source [(12)] in a normal-pressure atmosphere.

27. (amended) The apparatus of claim 26, wherein [Apparatus as set forth in one of claims 23 through 26 characterized in that] the electron beam source emits [(12) is adapted to emit] an electron beam with an electron energy of between 100 eV and 30 keV.

28. (amended) The apparatus of claim 27, further comprising [Apparatus as set forth in one of claims 23 through 27 characterized by spectral analysis] means for spectral analysis [(30) which are arranged and designed] to use light emitted by the converter material [(20.2; 34.3; 36.3; 38.3)] or the sample [(24)] for spectral analysis of the sample.

29. (amended) A production process for a sample holder [(20; 20'; 34; 36; 38)] for use in an apparatus [(10; 10')] for near-field optical imaging, in which a carrier [(20.1; 34.1; 36.1; 38.1,38.2)] is provided with a converter [(20.2; 34.2, 34.3; 36.3, 36.3; 38.3)] and in which the

carrier is then coated with an adhesion layer [(20.4; 34.5; 36.5; 38.5)], wherein the adhesion layer [(20.4; 34.5; 36.5; 38.5)] is applied in the form of mono- or bi-layers by attraction or by bursting vesicles.

30. (amended) The [A] process of [as set forth in] claim 29 further comprising [characterized by] a step of coating the carrier [(20.1; 34.1; 36.1; 38.1, 38.2)] with a protective layer [(20.3; 34.4; 36.4; 38.4)], in which a polyacryl is firstly applied as a monomer and then cross-linked by UV-irradiation to provide a two-dimensional mono-layer.

Please enter the following new claims:

31. (new) The sample holder of claim 1, wherein the adhesion layer thickness is constant over a portion of its lengthwise and transverse extent.

32. (new) The sample holder of claim 2, wherein the adhesion layer comprises a lipid or a cellulose derivative.

33. (new) The sample holder of claim 3, wherein the adhesion layer comprises a lipid or a cellulose derivative.

34. (new) The sample holder of claim 31, wherein the adhesion layer comprises a lipid or a cellulose derivative.

35. (new) The sample holder of claim 2, wherein the adhesion layer is a molecule mono-layer or molecule bi-layer.

36. (new) The sample holder of claim 34, wherein the adhesion layer is a molecule mono-layer or molecule bi-layer.

37. (new) The sample holder of claim 33, wherein the adhesion layer is a molecule mono-layer or molecule bi-layer.

38. (new) The sample holder of claim 4, wherein the adhesion layer is a molecule mono-layer or molecule bi-layer.

39. (new) The sample holder of claim 5, wherein the adhesion layer comprises a phospholipid.

40. (new) The sample holder of claim 35, wherein the adhesion layer comprises a phospholipid.

41. (new) The sample holder of claim 32, wherein the adhesion layer comprises a phospholipid.

42. (new) The sample holder of claim 36, wherein the adhesion layer comprises a phospholipid.

43. (new) The sample holder of claim 37, wherein the adhesion layer comprises a phospholipid.

44. (new) The sample holder of claim 5, wherein the adhesion layer contains a carboxylmetoxy cellulose.

45. (new) The sample holder of claim 40, wherein the adhesion layer contains a carboxylmetoxy cellulose.

46. (new) The sample holder of claim 41, wherein the adhesion layer contains a carboxylmetoxy cellulose.

47. (new) The sample holder of claim 42, wherein the adhesion layer contains a carboxylmetoxy cellulose.

48. (new) The sample holder of claim 43, wherein the adhesion layer contains a carboxylmetoxy cellulose.

49. (new) The sample holder of claim 39, wherein the adhesion layer contains a carboxylmetoxy cellulose.

50. (new) The sample holder of claim 47, wherein the converter material emits light of a spectral width of more than 50 nm wavelength upon electron irradiation.

51. (new) The sample holder of claim 50, wherein the converter material is embedded in small volumes in the carrier.

52. (new) The sample holder of claim 9, wherein the small volumes are regularly repetitively arranged at a small relative spacing in two directions.

53. (new) The sample holder of claim 52, wherein the converter material comprises phosphorus.

54. (new) The sample holder of claim 53, wherein the converter material comprises anthracene.

55. (new) The sample holder of claim 11, wherein the converter material contains micro- or nano-porous silicon.

56. (new) The sample holder of claim 12, wherein the converter material contains micro- or nano-porous silicon.

57. (new) The sample holder of claim 13, wherein the converter material contains micro- or nano-porous silicon.

58. (new) The sample holder of claim 8, wherein the converter material is homogenously distributed on the carrier.

59. (new) The sample holder of claim 11, wherein the converter material is homogenously distributed on the carrier.

60. (new) The sample holder of claim 12, wherein the converter material is homogenously distributed on the carrier.

61. (new) The sample holder of claim 13, wherein the converter material is homogenously distributed on the carrier.

62. (new) The sample holder of claim 14, wherein the converter material is homogenously distributed on the carrier.

63. (new) The sample holder of claim 17, wherein the semiconductor material is silicon.

64. (new) The sample holder of claim 17, wherein the semiconductor material is silicon dioxide.

65. (new) The sample holder of claim 17, wherein the semiconductor material is Si_3N_4.

66. (new) The sample holder of claim 17, wherein the semiconductor material is GaAs.

67. (new) The sample holder of claim 19, wherein the window comprises beryllium.

68. (new) The sample holder of claim 19, wherein the window comprises silicon.

69. (new) The sample holder of claim 54, further comprising a protective layer for protecting the converter from contamination by the sample, between the converter and the adhesion layer.

70. (new) The sample holder of claim 69, further comprising a nutrient solution container which is releasably fixed to the sample holder and which receives the sample and a nutrient solution.

71. (new) The apparatus of claim 23, wherein a deflection unit passes the electron beam in a scanning raster mode over a plane of the sample holder in which the converter material is arranged.

72. (new) The apparatus of claim 26, wherein the vacuum arrangement is such that a sample to be investigated is disposed on a side of the converter remote from the electron beam source in a normal-pressure atmosphere.

73. (new) The apparatus of claim 71, wherein the vacuum arrangement is such that a sample to be investigated is disposed on a side of the converter remote from the electron beam source in a normal-pressure atmosphere.

74. (new) The apparatus of claim 72, wherein the electron beam source emits an electron beam with an electron energy of between 100 eV and 30 keV.

75. (new) The apparatus of claim 73, wherein the electron beam source emits an electron beam with an electron energy of between 100 eV and 30 keV.

76. (new) The apparatus of claim 23, wherein the electron beam source emits an electron beam with an electron energy of between 100 eV and 30 keV.

77. (new) The apparatus of claim 23, further comprising means for spectral analysis to use light emitted by the converter material or the sample for spectral analysis of the sample.

REMARKS

In the specification, paragraph numbers and headings have been introduced, to comply the specification with standard US practice and to facilitate future amendment.

In the claims, multiple dependencies have been removed by distributing the limitations.

The above claims have also been amended to correspond them more closely to United States claiming practice, namely, by removing reference numerals, and by clarifying antecedent basis issues. In this manner, they should be in condition for allowance. These amendments to the claims are fully supported by the literal translation into English of the specification as filed in Germany, and they do not introduce new subject matter.

The claims as amended are incorporated into the substitute specification which is attached hereto.

Respectfully submitted,

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NEAR-FIELD OPTICAL INVESTIGATION APPARATUS

[0001] The invention relates to a sample holder, in particular for a biological sample, for use in an apparatus for near-field optical imaging, comprising a carrier and a converter which is connected to the carrier and which contains a converter material and is adapted to emit light of small lateral source extent upon irradiation with an electron beam.

BACKGROUND OF THE ART

[0002] The invention further concerns an apparatus for near-field optical imaging, having an electron beam source adapted to emit an electron beam and a sample holder.

[0003] Finally the invention concerns a production process for a sample holder for use in an apparatus for near-field optical imaging.

[0004] A multiplicity of different detector probes and detection modes are used for near-field optical microscopy. What is common to those methods is an operating procedure in which the probe is moved two-dimensionally (x-y-plane) and at each x-y-position the spacing of the probe relative to the sample (z-direction) must be set in accordance with a given criterion. Different interaction mechanisms are used for setting the spacing involved. The small spacing between the probe and the sample is necessary in order in the near field of the probe to achieve the high positional resolution, which can only be detected there, of such microscopy.

[0005] There exists a series of patents which include a near-field light source in the form of individual probes. In that respect there are descriptions of both detection by way of that aperture (DE 19531465; EP 0112401; US 5770885; US 5821409) and also a lighting arrangement (DE 19531802; DE 19714346). The design configuration of that nano light source with porous silicon (EP 0726480), fluorescing materials (DE 19504662; US 5105305) and drawn-out fibers (US 5286970) or by the excitation of excitons (US 5362963; US 5546223, US 5675433, US 5789742) is also described. Positioning of the individual probes is effected in the form of combined sensors for AFM and SNOM (DE 19631498; US 5354985; US 5821409), STM and SNOM (DE 4106548; US 5289004; US5770955), by way of shearing force detection (US 5548113, US 5641896) or by means of detected near-

field radiation (US 5304795, US 5666197). Use as a combined actuator and sensor is also described (US 5770856).

[0006] The disadvantage of those modes of procedure precisely in relation to biological samples lies in the necessary period of time for spacing adjustment, which makes up a large part of the overall measuring time. That gives rise to recording times in the region of several minutes. In that time, the living sample can move and change and thus give rise to defective imaging. In addition, the spacing measurement interaction is disturbed by an ordinary sample environment in the form of a nutrient solution, which can result in incorrect image data or even damage to the sample and the probe due to probe contact. All known methods use a light source in the form of a laser which only permits spectral narrow-band investigation of the sample.

[0007] US 5 633 972 describes a focused arrangement involving a plurality of drawn-out fibers with a spacing in respect of the individual sources of between 0.5 and 2 μm. The disadvantage of that arrangement is that it involves using a conventional process for approaching the multi-fiber probe to a sample surface. The change in the sample due to several hundreds of drawn-out fiber tips is to be considered critical, and likewise the chance of maintaining the near-field condition over the entire probe surface.

[0008] DE 196 01 109 A1 and WO97/25644 describe a two-dimensional arrangement of nano light sources on a flat substrate. The light sources are excited by way of high-energy radiation. That arrangement does not require any mechanically movable parts. It comprises a stationary plate with integrated near-field light sources. The sample is put on the plate. Here however a solid-state surface of semiconductor material affords a surface which is not suitable for biological samples. The samples do not assume either a fixed spacing or a constant position above the light sources. A further disadvantage is that the light sources are afforded by small cavities into which given materials are introduced. Those materials are contaminated by biological samples in nutrient solution. That prevents a stable mode of operation.

SUMMARY OF THE INVENTION

[0009] With that background in mind the object of the present invention is to provide a sample holder and an apparatus for near-field optical investigation of biological samples, which overcomes restrictions of the existing technologies. A

further object of the invention is to provide a production process for a sample holder of that kind.

[0010] In particular it is desirable for the apparatus to be suitable for the investigation of living biological samples and to permit sufficiently fast imaging so that the inherent movement of the sample or physiological changes can be observed. That requires image recording times in the seconds range or less and not in the minutes range, as is usual with the presentday technologies.

[0011] That object is attained by a sample holder of the kind set forth in the opening part of this specification, which has an adhesion layer which is arranged and adapted to fix the sample in the near field of the converter.

[0012] For an apparatus for near-field optical imaging, the object is attained by an arrangement having the features of claim 23.

[0013] In regard to the production process for a sample holder the object is attained by a process having the features of claim 29.

[0014] It has been found that biological objects, for example cells, can be stably fixed to a surface by an adhesion layer. By means of that adhesion layer on a support in which there are disposed a two-dimensional arrangement of nano light sources or one or more two-dimensionally displaceable nano light sources, it is possible to ensure constant maintenance of the near-field optical spacing between the sample and the light source.

[0015] Linked to the elimination of spacing regulation, as is necessary in the case of the probe processes, is a considerable saving of time which results in rapid image recording in the region of seconds or even less.

[0016] Shaping of the sample surface to the light source matrix is achieved by virtue of a suitable surface for the adhesion layer. The adhesion layer is disposed in the form of a thin layer (<30 nm) on the surface of the carrier and converter. The adhesion layer is preferably biocompatible. It is preferably made up of lipids, for example phospholipids, or cellulose derivatives, for example carboxylmetoxy cellulose.

[0017] In an embodiment of the invention the adhesion layer includes a phospholipid. A functional group can be deliberately attached to the phospholipid so as to afford a functional surface which only allows the addition of given groups which are suited thereto or enforces the orientation of molecules.

[0018] A constant layer thickness is important in terms of imaging. Therefore in a preferred embodiment the thickness of the adhesion layer is constant over a portion of its lengthwise and transverse extent. That portion includes the region in which the sample is connected to the adhesion layer. The adhesion layer is applied in the form of a mono- or bi-layer of molecules in order to achieve a constant thickness. That can be effected by attraction of the layer or also by bursting vesicles directly on the surface.

[0019] The necessary fixing of the sample with a constant spacing relative to the light sources is achieved by growing or adhering biological objects on that layer.

[0020] Upon excitation with electrons the converter material emits light of preferably relatively high intensity. In an embodiment of the invention the converter material is arranged in small volumes in the carrier layer. A series of materials are suitable for that purpose, which differ in terms of their yield, grain size and spectral emission. Phosphoruses, preferably materials with excitonic light emission such as anthracene or scintillator materials (crystallized out of solutions) such as for example BGO, are used.

[0021] In another embodiment the converter is applied in the form of a homogenous converter layer to the carrier or a carrier layer. In the preferred embodiment of the converter in the form of a homogenous converter layer, phosphoruses are preferably used, which are applied in the form of a thin coating to the carrier.

[0022] An alternative embodiment of the sample holder is distinguished in that the carrier forms the converter. In that case a carrier layer can also at the same time form the converter layer. The respective position of the light source in regard to image production is determined by the position of the electron beam. That has the advantage that the position of the light sources can be freely selected by suitable control of a per se known electron deflection unit.

[0023] In an embodiment, which is to emphasized, with patentability of its own, the converter material is produced by transformation of the carrier material or the material of a layer applied to the carrier.

[0024] In an embodiment in which the converter material contains transformed material of the carrier in small volumes, silicon is converted by etching into micro- or nanoporous silicon. Upon electron excitation micro- or nanoporous

silicon lights up very strongly in the optical range. Exciton decays are responsible for that. The decay energy and the life of the excitons can be influenced by the structural size, that is to say the pore size, of the porous silicon. With a smaller pore size, the band gap between valence and conduction band increases (quantum confinement). The luminescence of exciton decay therefore basically involves a correspondingly higher level of photon energy, the correspondingly smaller that the pore size is. Luminescence which originates from regions of smaller pores is therefore shifted in comparison with luminescence from regions of larger pores towards higher levels of photon energy. That is used to produce nano light sources in different spectral ranges (UV to NIR) by virtue of specific adjustment of different pore sizes or pore size ranges.

[0025] In a further embodiment in which the converter material is transformed the carrier material has small volumes which are deliberately doped with suitable impurities, by which intensive, strongly localized luminescences are produced upon electron beam excitation. If the carrier material involves a semiconductor, those luminescence effects can be produced by capture and recombination of charge carriers at the impurities or by the decay of localized excitons. Preferably in that respect the doped carrier material is a direct semiconductor such as GaAs. The doped carrier material may also involve a layer applied to the carrier.

[0026] In a further embodiment of the sample holder involving independent patentability the converter material used, upon electron irradiation, has a light emission with a spectral width of more than 50 nm wavelength. In that way the sample can be investigated in a comparatively wide spectral range. The production of a wider distribution of pore sizes provides for producing wide-band light sources (white light) which were not hitherto available in that form for near-field optical investigation procedures.

[0027] An enlargement in spectral emission can be achieved by utilizing per se known physical mechanisms of spectral enlargement. Such a mechanism is for example life enlargement. By deliberately adjusting the size of the excitation volume, it is possible for example to restrict the movement of the excitons, which can cause faster decay of the excitons produced after electron beam excitation. In accordance with the Heisenberg uncertainty principle the reduction in life results in a corresponding enlargement of energy distribution of the photons produced upon

exciton decay. Those possible options in terms of variation in spectral distribution and utilization of a wide spectral emission are used in accordance with the invention for spectral analysis of the samples.

[0028] For the first time that permits near-field optical spectroscopy on biological samples. It is possible, with a high level of positional resolution, to investigate optical properties such as transmission or luminescence of the sample in dependence on photon energy. With one recording it is possible to detect a multi-dimensional data field which is suitable for imaging purposes with high positional and with spectral resolution. In that way, the limitation to a small spectral band width, for example when using laser light sources, is overcome, and spectral information of the sample is also made available with a wide-band light source. The resolution in respect of time of image recording is improved with the approach according to the invention to such a degree that in addition development in respect of time of the properties of the sample can be detected by image recordings which are repeated at high frequency.

[0029] In a further embodiment the converter has an arrangement of small volumes of the converter material. The volumes are regularly repetitively arranged at small relative spacings in two directions.

[0030] In a further configuration of the concept of the invention the light sources are embedded in a carrier which is made from materials with silicon or gallium arsenide (GaAs). Those materials have the advantage that they are accessible to a wafer production process.

[0031] The carrier structure is preferably made up of Si, SiO_2 or Si_3N_4. Small volumes (transverse extent of less than 100 nm) of a converter material are introduced in that carrier or are produced therein by transformation (for example etching) of the carrier. Those volumes function as light sources and are preferably arranged in a two-dimensional grid raster at small spacings (smaller than 200 nm).

[0032] In a further embodiment for stabilization purposes the carrier is reinforced at spacings of between 10 and 100 μm by an additional thickening.

[0033] Alternatively or supplemental thereto, on the side towards an electron source of the carrier, an electron window can be included in the form of a plane-parallel plate with a low level of absorption for electrons. It comprises beryllium or silicon. The window is preferably of such a configuration and arrangement that it reinforces the carrier.

[0034] A further embodiment of the sample holder according to the invention includes a protective layer between the converter and the adhesion layer, which is adapted to protect the converter from contamination by the sample. It has been found that contamination of the light sources is prevented by the additional production of a protective layer between the light source layer and the adhesion layer. Preferably thin (< 20 nm) plastic films are used here, preferably polyacryl, which is applied in the form of a monomer and which is cross-linked by UV-irradiation to form two-dimensional mono-layers.

[0035] In dependence on the sample to be investigated and the nutrient medium the adhesion layer can already afford an adequate protection function so that the protective layer can also be omitted in the case of suitable samples.

[0036] A further embodiment of the sample holder according to the invention has a nutrient solution container which is adapted to receive the sample and a nutrient solution. The nutrient solution container is open towards the adhesion layer and thus permits fixing of the sample to the adhesion layer. Contamination of the apparatus with nutrient solution is avoided by means of that container. Preferably the container and the sample holder are such that the container can be selectively fitted or removed, depending on the respective nature of the sample.

[0037] The apparatus according to the invention for near-field optical imaging with an electron beam source adapted to emit an electron beam and a sample holder permits particularly fast imaging, in particular when investigating biological samples, by virtue of the sample holder according to the invention which is provided in the apparatus.

[0038] A preferred embodiment is such that the converter can be excited in vacuum by the electron beam.

[0039] In a further embodiment of the invention the vacuum arrangement required for that purpose is such that a sample to be investigated is disposed on the side of the converter, which is remote from the electron beam source, in an atmosphere at normal pressure.

[0040] The apparatus and the sample holder are preferably so designed that the sample holder can be removed when no vacuum is applied. The measures which are required for that purpose, in particular in the field of vacuum technology, are known per se.

[0041] In the case of samples which can be exposed to vacuum, it is also possible to implement measurement in an "incident-light arrangement". The converter is illuminated by the electron beam, with the electron beam passing through the sample. The light which is scattered or diffracted or emitted in a lateral direction can be used for optical imaging purposes. In that case the structure comprising the window, the carrier and the converter can be simplified so that the structures are implemented in a greater thickness and the window is eliminated. Image contrast then comprises a combination of electron beam absorption/scatter and optical absorption.

[0042] In a further preferred embodiment of the apparatus according to the invention the electron beam can be passed in scanning raster form over the plane in which the converter material is arranged. Provided for that purpose is a suitable electron optical system which includes a deflection unit and a focusing electron lens. The technology required for raster scanning of the converter is known for example from scanning electron microscopes or scanning cathodoluminescence arrangements.

[0043] The optical signal is picked up with a converging optical system and a photodetector which is arranged in the far field of the light source. A near-field microscopic image is gradually produced by sequential excitation of the light sources at various positions and representation of the signal intensity in dependence on the light source position. Image contrast essentially comprises the object contrast in the near field of the light sources. In this respect the contrast may involve light absorption which is dependent on the respectively through-radiated region within the sample, or also fluorescence of the sample, which differs in dependence on location.

[0044] In a particularly preferred embodiment the apparatus according to the invention includes spectral analysis means which are arranged and designed in such a way as to use light emitted by the converter material or by a sample, for spectral analysis of the sample. The light guide, imaging and spectral analysis units which are required for that purpose and which go beyond the converging optical system and which in particular must be designed for spectrally resolved detection of weak light signals are known per se. In this case also reference may be made by way of example to scanning cathodoluminescence.

[0045] The electron beam source is adapted to emit an electron beam with an electron energy of between 100 eV and 30 keV.

[0046] Further embodiments each involving patentability of their own, instead of an electron beam source, use other particle sources or electromagnetic radiation for the excitation of light emissions of the converter, in particular in the UV, VUV, X-ray or gamma spectral ranges.

[0047] In the production process according to the invention for a sample holder for use in an apparatus for near-field optical imaging, in which a carrier is provided with a converter and in which there is effected a step of coating the carrier provided with the converter with an adhesion layer, the adhesion layer is applied in the form of mono- or bi-layers by attraction or by bursting vesicles on the surface. That technology makes it possible to produce the adhesion layer in the particularly small thickness required for near-field microscopy, of less than a hundred nm. At the same time that procedure provides constant layer thicknesses which are important in order to position the samples at a constant spacing relative to the light sources.

[0048] In an embodiment of the process according to the invention, there is a step of coating the carrier with a protective layer. Such a protective layer is preferably made of polyacryl and applied in the form of a monomer and cross-linked by UV-irradiation to form a two-dimensional mono-layer. The thickness is 20 nm at a maximum.

BRIEF DESCRIPTION OF THE INVENTION

[0049] Further features and advantages of the invention are more clearly set forth hereinafter by the description of some embodiments with reference to the drawing in which:

[0050] Figure 1 shows a first embodiment of an apparatus according to the invention for near-field optical imaging,

[0051] Figure 2 shows a modification of the embodiment of Figure 1, with which samples which can be exposed to a vacuum are investigated,

[0052] Figure 3 is a diagrammatic view in cross-section of a first embodiment of the sample holder according to the invention,

[0053] Figure 4 is a corresponding view showing a second embodiment of the sample holder according to the invention,

[0054] Figure 5 is a view in cross-section, also in greatly simplified form, of a third embodiment of the sample holder according to the invention, and

[0055] Figure 6 shows a fourth embodiment of the sample holder according to the invention, as an alternative to the embodiment of Figure 3.

DETAILED DESCRIPTION OF THE INVENTION

[0056] Figure 1 shows a first embodiment of an apparatus 10 according to the invention for near-field optical imaging, as a greatly simplified cross-sectional view. An electron beam source 12 produces an electron beam 13 which is indicated by boundary lines and which on its way passes through a deflection unit 14 and an electron-optical lens 16. The entire electron optical system of the apparatus is disposed in a vacuum chamber 18 which can be evacuated and filled with air by devices which are not shown here. The electron beam impinges with its focus on a sample holder 20 whose properties are described in greater detail hereinafter with reference to Figures 3 through 6. The electron beam can be deflected by means of the deflection unit 14 in two orthogonal directions perpendicularly to the beam direction, independently of each other, so that the electron beam focus can be passed in a scanning raster form over a converter layer (see Figures 3 through 6) of the sample holder 20 and nano light sources 22 contained in the converter layer excited to light up. The light emitted in different directions by the light source 22 is indicated by arrows in Figure 1.

[0057] While the electron beam guide means is disposed completely in the vacuum, a sample 24, on the side of the sample holder which is remote from the electron beam, is arranged under normal pressure. The sample is stably fixed on the sample holder 20 at the near-field spacing by an adhesion layer (not shown here). The light produced by non-elastic electron scatter by the nano light source 22 in the converter passes through the sample 24 and is collected in the optical far field by a high-aperture detection optical means 26. The beam path is indicated by boundary lines 28. The image of the captured light signal is produced on a light-sensitive layer of a photodetector 30, converted into an electrical signal corresponding to the intensity thereof, and fed to an image producing and image processing unit (not shown). In the view which is not to scale in Figure 1, the sample 24 and the sample holder 20 are shown on a greatly enlarged scale.

[0058] Figure 2 shows a modified arrangement 10' of the embodiment of Figure 1 for samples which can be exposed to vacuum. The same references denote the same components, in comparison with the foregoing example of Figure 1. In the present embodiment, the sample 24 is arranged in the vacuum chamber 18 and has the electron beam 13 passing therethrough. The sample 24 is fixed on a sample holder 20'. Unlike Figure 1, in this embodiment the sample holder 20' can be thicker. The sample holder 20' otherwise differs from the sample holder 20 in Figure 1 in that its adhesion layer is arranged on the side which is towards the electron source.

[0059] The electron beam 13 causes excitation of a nano light source 22 for the emission of light which passes through the sample and the image of which is formed laterally on the detector 20 through a high-aperture objective lens 26'.

[0060] Figure 3 shows a first preferred embodiment of a sample holder 20 according to the invention. The electron beam 13 which comes from below in the drawing firstly passes through an electron window 32, then penetrates a carrier layer 20.1 on which a converter layer 20.2 is disposed. In the converter layer 20.2 the electron beam 13 is greatly retarded by non-elastic scatter. The energy transmitted to the converter material excites it to emit light. The region within the converter, in which the electrons are retarded represents the nano light source 22.

[0061] The converter layer 20.2 is protected from contamination and oxidation by a protective layer 20.3. Disposed in turn on the protective layer 20.3 is an adhesion layer 20.4 which serves for mounting the sample 24. Only the part of the sample 24 which is in the near field of the light source contributes substantially to the optical signal which is measured in the far field.

[0062] In the present embodiment the sample 24 is arranged in a nutrient solution container 34 which contains a nutrient solution indicated by broken-line hatching.

[0063] The position of the nano light source 22 and therewith simultaneously the position of the sample at which measurements are taken are altered by movement of the focus of the electron beam 13 in the plane of the converter layer 20.2. The near-field image of the sample is obtained by the representation of the intensity of the optical signal, in dependence on the location of the light source.

[0064] Figure 4 shows a sample holder 34, as an alternative embodiment of the invention. The electron beam 13 which comes from below in the drawing

passes through a carrier layer 34.1. Arranged in the upper region of the carrier layer 34.1 in small volumes 34.2 is a converter material 34.3 which is produced by transformation of the carrier material. This involves microporous silicon. The converter material 34.3 is excited to produce intensive light by virtue of electron beam excitation with the electron beam. A protective layer 34.4 protects the microporous silicon 34.3 from contamination. The protective layer is in turn covered by an adhesion layer 34.5 which serves for fixing the sample 24 (not shown here). Otherwise the further structure of the sample holder can be identical to the description with reference to Figure 3.

[0065] Figure 5 shows a sample holder 36 with a carrier structure 36.1, as a further embodiment of the invention. Unlike the sample holder 34 shown in Figure 4, provided in the carrier structure 36.1 are conical holes 36.2, in which a converter material 36.3 is crystallized out by evaporation of the solvent of a solution of anthracene or scintillators. Upon electron beam excitation the converter material 36.3 intensively emits light and is used as described with reference to Figure 3 for imaging purposes.

[0066] Figure 6 shows a sample holder 38 as an embodiment as an alternative to the structure shown in Figure 3. A particularly thin carrier matrix 38.1 is made possible by virtue of the fact that it is reinforced by support structures 38.2 which are spaced from each other at a relatively large spacing of between 10 and 100 μm. The support structures carry the forces of the pressure difference between the vacuum on the side towards the electron source 12 (Figure 1) and the normal pressure on the side remote from the electron source 12. The particularly thin carrier layer affords the advantage that the electron beam 13 is only slightly scattered. In that way the extent of the nano light source 22 excited to produce light (see Figure 1) is reduced, whereby ultimately the lateral resolution of the near-field microscope is increased.

CLAIMS

What is claimed is:

1. A sample holder, in particular for a biological sample, for use in an apparatus for near-field optical imaging, comprising :

a carrier and

a converter which is connected to the carrier and which contains a converter material and emits light of small lateral source extent upon irradiation with an electron beam, characterized by an adhesion layer which fixes the sample in the near field of the converter.

2. The sample holder of claim 1, wherein the adhesion layer has a thickness of a maximum of 30 nm.

3. The sample holder of claim 1, wherein the adhesion layer thickness is constant over a portion of its lengthwise and transverse extent.

4. The sample holder of claim 1, wherein the adhesion layer comprises a lipid or a cellulose derivative.

5. The sample holder of claim 1, wherein the adhesion layer is a molecule mono-layer or molecule bi-layer.

6. The sample holder of claim 4, wherein the adhesion layer comprises a phospholipid.

7. The sample holder of claim 4, wherein the adhesion layer contains a carboxylmetoxy cellulose.

8. The sample holder of claim 1, wherein the converter material emits light of a spectral width of more than 50 nm wavelength upon electron irradiation.

9. The sample holder of claim 1, wherein the converter material is embedded in small volumes in the carrier.

10. The sample holder of claim 9, wherein the small volumes are regularly repetitively arranged at a small relative spacing in two directions.

11. The sample holder of claim 1, wherein the converter material comprises phosphorus.

12. The sample holder of claim 1, wherein the converter material comprises anthracene.

13. The sample holder of claim 1, wherein the converter material comprises transformed material of the carrier in small volumes.

14. The sample holder of claim 1, wherein the converter material contains micro- or nano-porous silicon.

15. The sample holder of claim 1, wherein the converter material is homogenously distributed on the carrier.

16. The sample holder of claim 14, wherein the carrier forms the converter.

17. The sample holder of claim 1, wherein the carrier comprises a semiconductor material.

18. The sample holder of claim 1, wherein the carrier is reinforced at spacings of between 10 and 100 μm by an additional thickening.

19. The sample holder of claim 1, further comprising a window which is transmissive for electrons, on the side of the carrier which is remote from the adhesion layer.

20. The sample holder of claim 19, wherein the window reinforces the carrier.

21. The sample holder of claim 1, further comprising a protective layer for protecting the converter from contamination by the sample, between the converter and the adhesion layer.

22. The sample holder of claim 1, further comprising a nutrient solution container which is releasably fixed to the sample holder and which receives the sample and a nutrient solution.

23. An apparatus for near-field optical imaging comprising:

a source for emitting an electron beam; and

a sample holder, comprising:

a carrier and

a converter that is connected to the carrier and that contains a converter material that emits light of small lateral source extent upon irradiation with the electron beam, characterized by an adhesion layer that fixes the sample in the near field of the converter.

24. The apparatus of claim 23, further comprising:

a vacuum arrangement such that the converter can be excited in vacuum by the electron beam.

25. The apparatus of claim 24, wherein a deflection unit passes the electron beam in a scanning raster mode over a plane of the sample holder in which the converter material is arranged.

26. The apparatus of claim 25, wherein the vacuum arrangement is such that a sample to be investigated is disposed on a side of the converter remote from the electron beam source in a normal-pressure atmosphere.

27. The apparatus of claim 26, wherein the electron beam source emits an electron beam with an electron energy of between 100 eV and 30 keV.

28. The apparatus of claim 27, further comprising:

means for spectral analysis to use light emitted by the converter material or the sample for spectral analysis of the sample.

29. A production process for a sample holder for use in an apparatus for near-field optical imaging, in which a carrier is provided with a converter and in which the carrier is then coated with an adhesion layer, wherein the adhesion layer is applied in the form of mono- or bi-layers by attraction or by bursting vesicles.

30. The process of claim 29 further comprising a step of coating the carrier with a protective layer, in which a polyacryl is firstly applied as a monomer and then cross-linked by UV-irradiation to provide a two-dimensional mono-layer.

31. The sample holder of claim 1, wherein the adhesion layer thickness is constant over a portion of its lengthwise and transverse extent.

32. The sample holder of claim 2, wherein the adhesion layer comprises a lipid or a cellulose derivative.

33. The sample holder of claim 3, wherein the adhesion layer comprises a lipid or a cellulose derivative.

34. The sample holder of claim 31, wherein the adhesion layer comprises a lipid or a cellulose derivative.

35. The sample holder of claim 2, wherein the adhesion layer is a molecule mono-layer or molecule bi-layer.

36. The sample holder of claim 34, wherein the adhesion layer is a molecule mono-layer or molecule bi-layer.

37. The sample holder of claim 33, wherein the adhesion layer is a molecule mono-layer or molecule bi-layer.

38. The sample holder of claim 4, wherein the adhesion layer is a molecule mono-layer or molecule bi-layer.

39. The sample holder of claim 5, wherein the adhesion layer comprises a phospholipid.

40. The sample holder of claim 35, wherein the adhesion layer comprises a phospholipid.

41. The sample holder of claim 32, wherein the adhesion layer comprises a phospholipid.

42. The sample holder of claim 36, wherein the adhesion layer comprises a phospholipid.

43. The sample holder of claim 37, wherein the adhesion layer comprises a phospholipid.

44. The sample holder of claim 5, wherein the adhesion layer contains a carboxylmetoxy cellulose.

45. The sample holder of claim 40, wherein the adhesion layer contains a carboxylmetoxy cellulose.

46. The sample holder of claim 41, wherein the adhesion layer contains a carboxylmetoxy cellulose.

47. The sample holder of claim 42, wherein the adhesion layer contains a carboxylmetoxy cellulose.

48. The sample holder of claim 43, wherein the adhesion layer contains a carboxylmetoxy cellulose.

49. The sample holder of claim 39, wherein the adhesion layer contains a carboxylmetoxy cellulose.

50. The sample holder of claim 47, wherein the converter material emits light of a spectral width of more than 50 nm wavelength upon electron irradiation.

51. The sample holder of claim 50, wherein the converter material is embedded in small volumes in the carrier.

52. The sample holder of claim 9, wherein the small volumes are regularly repetitively arranged at a small relative spacing in two directions.

53. The sample holder of claim 52, wherein the converter material comprises phosphorus.

54. The sample holder of claim 53, wherein the converter material comprises anthracene.

55. The sample holder of claim 11, wherein the converter material contains micro- or nano-porous silicon.

56. The sample holder of claim 12, wherein the converter material contains micro- or nano-porous silicon.

57. The sample holder of claim 13, wherein the converter material contains micro- or nano-porous silicon.

58. The sample holder of claim 8, wherein the converter material is homogenously distributed on the carrier.

59. The sample holder of claim 11, wherein the converter material is homogenously distributed on the carrier.

60. The sample holder of claim 12, wherein the converter material is homogenously distributed on the carrier.

61. The sample holder of claim 13, wherein the converter material is homogenously distributed on the carrier.

62. The sample holder of claim 14, wherein the converter material is homogenously distributed on the carrier.

63. The sample holder of claim 17, wherein the semiconductor material is silicon.

64. The sample holder of claim 17, wherein the semiconductor material is silicon dioxide.

65. The sample holder of claim 17, wherein the semiconductor material is Si_3N_4.

66. The sample holder of claim 17, wherein the semiconductor material is GaAs.

67. The sample holder of claim 19, wherein the window comprises beryllium.

68. The sample holder of claim 19, wherein the window comprises silicon.

69. The sample holder of claim 54, further comprising a protective layer for protecting the converter from contamination by the sample, between the converter and the adhesion layer.

70. The sample holder of claim 69, further comprising a nutrient solution container which is releasably fixed to the sample holder and which receives the sample and a nutrient solution.

71. The apparatus of claim 23, wherein a deflection unit passes the electron beam in a scanning raster mode over a plane of the sample holder in which the converter material is arranged.

72. The apparatus of claim 26, wherein the vacuum arrangement is such that a sample to be investigated is disposed on a side of the converter remote from the electron beam source in a normal-pressure atmosphere.

73. The apparatus of claim 71, wherein the vacuum arrangement is such that a sample to be investigated is disposed on a side of the converter remote from the electron beam source in a normal-pressure atmosphere.

74. The apparatus of claim 72, wherein the electron beam source emits an electron beam with an electron energy of between 100 eV and 30 keV.

75. The apparatus of claim 73, wherein the electron beam source emits an electron beam with an electron energy of between 100 eV and 30 keV.

76. The apparatus of claim 23, wherein the electron beam source emits an electron beam with an electron energy of between 100 eV and 30 keV.

77. The apparatus of claim 23, further comprising means for spectral analysis to use light emitted by the converter material or the sample for spectral analysis of the sample.

Abstract

The invention concerns a sample holder (20), in particular for a biological sample (24), for use in an apparatus for near-field optical imaging, comprising a carrier (20.1) and a converter (20.2) which is connected to the carrier and which contains a converter material and is adapted to emit light of small lateral source extent upon irradiation with an electron beam (13). In accordance with the invention there is provided an adhesion layer (20.4) which is arranged and adapted to fix the sample (24) in the near field of the converter (20.2).

Figure 3

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Near-field optical investigation apparatus

The invention relates to a sample holder, in particular for a biological sample, for use in an apparatus for near-field optical imaging, comprising a carrier and a converter which is connected to the carrier and which contains a converter material and is adapted to emit light of small lateral source extent upon irradiation with an electron beam.

The invention further concerns an apparatus for near-field optical imaging, having an electron beam source adapted to emit an electron beam and a sample holder.

Finally the invention concerns a production process for a sample holder for use in an apparatus for near-field optical imaging.

A multiplicity of different detector probes and detection modes are used for near-field optical microscopy. What is common to those methods is an operating procedure in which the probe is moved two-dimensionally (x-y-plane) and at each x-y-position the spacing of the probe relative to the sample (z-direction) must be set in accordance with a given criterion. Different interaction mechanisms are used for setting

the spacing involved. The small spacing between the probe and the sample is necessary in order in the near field of the probe to achieve the high positional resolution, which can only be detected there, of such microscopy.

There exists a series of patents which include a near-field light source in the form of individual probes. In that respect there are descriptions of both detection by way of that aperture (DE 19531465; EP 0112401; US 5770885; US 5821409) and also a lighting arrangement (DE 19531802; DE 19714346). The design configuration of that nano light source with porous silicon (EP 0726480), fluorescing materials (DE 19504662; US 5105305) and drawn-out fibers (US 5286970) or by the excitation of excitons (US 5362963; US 5546223, US 5675433, US 5789742) is also described. Positioning of the individual probes is effected in the form of combined sensors for AFM and SNOM (DE 19631498; US 5354985; US 5821409), STM and SNOM (DE 4106548; US 5289004; US5770955), by way of shearing force detection (US 5548113, US 5641896) or by means of detected near-field radiation (US 5304795, US 5666197). Use as a combined actuator and sensor is also described (US 5770856).

The disadvantage of those modes of procedure precisely in relation to biological samples lies in the necessary period of time for spacing adjustment, which makes up a large part of the overall measuring time. That gives rise to recording times in the region of several minutes. In that time, the living sample can move and change and thus give rise to defective imaging. In addition, the spacing measurement interaction is disturbed by an ordinary sample environment in the form of a nutrient solution, which can result in incorrect image data or even damage to the sample and the probe due to probe contact. All known methods use a light source in the form of a laser which only permits spectral narrow-band investigation of the sample.

US 5 633 972 describes a focused arrangement involving a plurality of drawn-out fibers with a spacing in respect of the individual sources of

between 0.5 and 2 μm. The disadvantage of that arrangement is that it involves using a conventional process for approaching the multi-fiber probe to a sample surface. The change in the sample due to several hundreds of drawn-out fiber tips is to be considered critical, and likewise the chance of maintaining the near-field condition over the entire probe surface.

DE 196 01 109 A1 and WO97/25644 describe a two-dimensional arrangement of nano light sources on a flat substrate. The light sources are excited by way of high-energy radiation. That arrangement does not require any mechanically movable parts. It comprises a stationary plate with integrated near-field light sources. The sample is put on the plate. Here however a solid-state surface of semiconductor material affords a surface which is not suitable for biological samples. The samples do not assume either a fixed spacing or a constant position above the light sources. A further disadvantage is that the light sources are afforded by small cavities into which given materials are introduced. Those materials are contaminated by biological samples in nutrient solution. That prevents a stable mode of operation.

With that background in mind the object of the present invention is to provide a sample holder and an apparatus for near-field optical investigation of biological samples, which overcomes restrictions of the existing technologies. A further object of the invention is to provide a production process for a sample holder of that kind.

In particular it is desirable for the apparatus to be suitable for the investigation of living biological samples and to permit sufficiently fast imaging so that the inherent movement of the sample or physiological changes can be observed. That requires image recording times in the seconds range or less and not in the minutes range, as is usual with the presentday technologies.

That object is attained by a sample holder of the kind set forth in the opening part of this specification, which has an adhesion layer which is arranged and adapted to fix the sample in the near field of the converter.

For an apparatus for near-field optical imaging, the object is attained by an arrangement having the features of claim 23.

In regard to the production process for a sample holder the object is attained by a process having the features of claim 29.

It has been found that biological objects, for example cells, can be stably fixed to a surface by an adhesion layer. By means of that adhesion layer on a support in which there are disposed a two-dimensional arrangement of nano light sources or one or more two-dimensionally displaceable nano light sources, it is possible to ensure constant maintenance of the near-field optical spacing between the sample and the light source.

Linked to the elimination of spacing regulation, as is necessary in the case of the probe processes, is a considerable saving of time which results in rapid image recording in the region of seconds or even less.

Shaping of the sample surface to the light source matrix is achieved by virtue of a suitable surface for the adhesion layer. The adhesion layer is disposed in the form of a thin layer (<30 nm) on the surface of the carrier and converter. The adhesion layer is preferably biocompatible. It is preferably made up of lipids, for example phospholipids, or cellulose derivatives, for example carboxylmetoxy cellulose.

In an embodiment of the invention the adhesion layer includes a phospholipid. A functional group can be deliberately attached to the phospholipid so as to afford a functional surface which only allows the addition of given groups which are suited thereto or enforces the orientation of molecules.

A constant layer thickness is important in terms of imaging. Therefore in a preferred embodiment the thickness of the adhesion layer is constant over a portion of its lengthwise and transverse extent. That portion includes the region in which the sample is connected to the adhesion layer. The adhesion layer is applied in the form of a mono- or bi-layer of molecules in order to achieve a constant thickness. That can

be effected by attraction of the layer or also by bursting vesicles directly on the surface.

The necessary fixing of the sample with a constant spacing relative to the light sources is achieved by growing or adhering biological objects on that layer.

Upon excitation with electrons the converter material emits light of preferably relatively high intensity. In an embodiment of the invention the converter material is arranged in small volumes in the carrier layer. A series of materials are suitable for that purpose, which differ in terms of their yield, grain size and spectral emission. Phosphoruses, preferably materials with excitonic light emission such as anthracene or scintillator materials (crystallized out of solutions) such as for example BGO, are used.

In another embodiment the converter is applied in the form of a homogenous converter layer to the carrier or a carrier layer. In the preferred embodiment of the converter in the form of a homogenous converter layer, phosphoruses are preferably used, which are applied in the form of a thin coating to the carrier.

An alternative embodiment of the sample holder is distinguished in that the carrier forms the converter. In that case a carrier layer can also at the same time form the converter layer. The respective position of the light source in regard to image production is determined by the position of the electron beam. That has the advantage that the position of the light sources can be freely selected by suitable control of a per se known electron deflection unit.

In an embodiment, which is to emphasized, with patentability of its own, the converter material is produced by transformation of the carrier material or the material of a layer applied to the carrier.

In an embodiment in which the converter material contains transformed material of the carrier in small volumes, silicon is converted by etching into micro- or nanoporous silicon. Upon electron excitation micro- or nanoporous silicon lights up very strongly in the optical range.

Exciton decays are responsible for that. The decay energy and the life of the excitons can be influenced by the structural size, that is to say the pore size, of the porous silicon. With a smaller pore size, the band gap between valence and conduction band increases (quantum confinement). The luminescence of exciton decay therefore basically involves a correspondingly higher level of photon energy, the correspondingly smaller that the pore size is. Luminescence which originates from regions of smaller pores is therefore shifted in comparison with luminescence from regions of larger pores towards higher levels of photon energy. That is used to produce nano light sources in different spectral ranges (UV to NIR) by virtue of specific adjustment of different pore sizes or pore size ranges.

In a further embodiment in which the converter material is transformed the carrier material has small volumes which are deliberately doped with suitable impurities, by which intensive, strongly localized luminescences are produced upon electron beam excitation. If the carrier material involves a semiconductor, those luminescence effects can be produced by capture and recombination of charge carriers at the impurities or by the decay of localized excitons. Preferably in that respect the doped carrier material is a direct semiconductor such as GaAs. The doped carrier material may also involve a layer applied to the carrier.

In a further embodiment of the sample holder involving independent patentability the converter material used, upon electron irradiation, has a light emission with a spectral width of more than 50 nm wavelength. In that way the sample can be investigated in a comparatively wide spectral range. The production of a wider distribution of pore sizes provides for producing wide-band light sources (white light) which were not hitherto available in that form for near-field optical investigation procedures.

An enlargement in spectral emission can be achieved by utilizing per se known physical mechanisms of spectral enlargement. Such a mechanism is for example life enlargement. By deliberately adjusting the

size of the excitation volume, it is possible for example to restrict the movement of the excitons, which can cause faster decay of the excitons produced after electron beam excitation. In accordance with the Heisenberg uncertainty principle the reduction in life results in a corresponding enlargement of energy distribution of the photons produced upon exciton decay. Those possible options in terms of variation in spectral distribution and utilization of a wide spectral emission are used in accordance with the invention for spectral analysis of the samples.

For the first time that permits near-field optical spectroscopy on biological samples. It is possible, with a high level of positional resolution, to investigate optical properties such as transmission or luminescence of the sample in dependence on photon energy. With one recording it is possible to detect a multi-dimensional data field which is suitable for imaging purposes with high positional and with spectral resolution. In that way, the limitation to a small spectral band width, for example when using laser light sources, is overcome, and spectral information of the sample is also made available with a wide-band light source. The resolution in respect of time of image recording is improved with the approach according to the invention to such a degree that in addition development in respect of time of the properties of the sample can be detected by image recordings which are repeated at high frequency.

In a further embodiment the converter has an arrangement of small volumes of the converter material. The volumes are regularly repetitively arranged at small relative spacings in two directions.

In a further configuration of the concept of the invention the light sources are embedded in a carrier which is made from materials with silicon or gallium arsenide (GaAs). Those materials have the advantage that they are accessible to a wafer production process.

The carrier structure is preferably made up of Si, SiO_2 or Si_3N_4. Small volumes (transverse extent of less than 100 nm) of a converter material are introduced in that carrier or are produced therein by transformation (for example etching) of the carrier. Those volumes

function as light sources and are preferably arranged in a two-dimensional grid raster at small spacings (smaller than 200 nm).

In a further embodiment for stabilization purposes the carrier is reinforced at spacings of between 10 and 100 μm by an additional thickening.

Alternatively or supplemental thereto, on the side towards an electron source of the carrier, an electron window can be included in the form of a plane-parallel plate with a low level of absorption for electrons. It comprises beryllium or silicon. The window is preferably of such a configuration and arrangement that it reinforces the carrier.

A further embodiment of the sample holder according to the invention includes a protective layer between the converter and the adhesion layer, which is adapted to protect the converter from contamination by the sample. It has been found that contamination of the light sources is prevented by the additional production of a protective layer between the light source layer and the adhesion layer. Preferably thin (< 20 nm) plastic films are used here, preferably polyacryl, which is applied in the form of a monomer and which is cross-linked by UV-irradiation to form two-dimensional mono-layers.

In dependence on the sample to be investigated and the nutrient medium the adhesion layer can already afford an adequate protection function so that the protective layer can also be omitted in the case of suitable samples.

A further embodiment of the sample holder according to the invention has a nutrient solution container which is adapted to receive the sample and a nutrient solution. The nutrient solution container is open towards the adhesion layer and thus permits fixing of the sample to the adhesion layer. Contamination of the apparatus with nutrient solution is avoided by means of that container. Preferably the container and the sample holder are such that the container can be selectively fitted or removed, depending on the respective nature of the sample.

The apparatus according to the invention for near-field optical imaging with an electron beam source adapted to emit an electron beam and a sample holder permits particularly fast imaging, in particular when investigating biological samples, by virtue of the sample holder according to the invention which is provided in the apparatus.

A preferred embodiment is such that the converter can be excited in vacuum by the electron beam.

In a further embodiment of the invention the vacuum arrangement required for that purpose is such that a sample to be investigated is disposed on the side of the converter, which is remote from the electron beam source, in an atmosphere at normal pressure.

The apparatus and the sample holder are preferably so designed that the sample holder can be removed when no vacuum is applied. The measures which are required for that purpose, in particular in the field of vacuum technology, are known per se.

In the case of samples which can be exposed to vacuum, it is also possible to implement measurement in an "incident-light arrangement". The converter is illuminated by the electron beam, with the electron beam passing through the sample. The light which is scattered or diffracted or emitted in a lateral direction can be used for optical imaging purposes. In that case the structure comprising the window, the carrier and the converter can be simplified so that the structures are implemented in a greater thickness and the window is eliminated. Image contrast then comprises a combination of electron beam absorption/scatter and optical absorption.

In a further preferred embodiment of the apparatus according to the invention the electron beam can be passed in scanning raster form over the plane in which the converter material is arranged. Provided for that purpose is a suitable electron optical system which includes a deflection unit and a focusing electron lens. The technology required for raster scanning of the converter is known for example from scanning electron microscopes or scanning cathodoluminescence arrangements.

The optical signal is picked up with a converging optical system and a photodetector which is arranged in the far field of the light source. A near-field microscopic image is gradually produced by sequential excitation of the light sources at various positions and representation of the signal intensity in dependence on the light source position. Image contrast essentially comprises the object contrast in the near field of the light sources. In this respect the contrast may involve light absorption which is dependent on the respectively through-radiated region within the sample, or also fluorescence of the sample, which differs in dependence on location.

In a particularly preferred embodiment the apparatus according to the invention includes spectral analysis means which are arranged and designed in such a way as to use light emitted by the converter material or by a sample, for spectral analysis of the sample. The light guide, imaging and spectral analysis units which are required for that purpose and which go beyond the converging optical system and which in particular must be designed for spectrally resolved detection of weak light signals are known per se. In this case also reference may be made by way of example to scanning cathodoluminescence.

The electron beam source is adapted to emit an electron beam with an electron energy of between 100 eV and 30 keV.

Further embodiments each involving patentability of their own, instead of an electron beam source, use other particle sources or electromagnetic radiation for the excitation of light emissions of the converter, in particular in the UV, VUV, X-ray or gamma spectral ranges.

In the production process according to the invention for a sample holder for use in an apparatus for near-field optical imaging, in which a carrier is provided with a converter and in which there is effected a step of coating the carrier provided with the converter with an adhesion layer, the adhesion layer is applied in the form of mono- or bi-layers by attraction or by bursting vesicles on the surface. That technology makes it possible to produce the adhesion layer in the particularly small thickness required for

near-field microscopy, of less than a hundred nm. At the same time that procedure provides constant layer thicknesses which are important in order to position the samples at a constant spacing relative to the light sources.

In an embodiment of the process according to the invention, there is a step of coating the carrier with a protective layer. Such a protective layer is preferably made of polyacryl and applied in the form of a monomer and cross-linked by UV-irradiation to form a two-dimensional mono-layer. The thickness is 20 nm at a maximum.

Further features and advantages of the invention are more clearly set forth hereinafter by the description of some embodiments with reference to the drawing in which:

Figure 1 shows a first embodiment of an apparatus according to the invention for near-field optical imaging,

Figure 2 shows a modification of the embodiment of Figure 1, with which samples which can be exposed to a vacuum are investigated,

Figure 3 is a diagrammatic view in cross-section of a first embodiment of the sample holder according to the invention,

Figure 4 is a corresponding view showing a second embodiment of the sample holder according to the invention,

Figure 5 is a view in cross-section, also in greatly simplified form, of a third embodiment of the sample holder according to the invention, and

Figure 6 shows a fourth embodiment of the sample holder according to the invention, as an alternative to the embodiment of Figure 3.

Figure 1 shows a first embodiment of an apparatus 10 according to the invention for near-field optical imaging, as a greatly simplified cross-sectional view. An electron beam source 12 produces an electron beam 13 which is indicated by boundary lines and which on its way passes through a deflection unit 14 and an electron-optical lens 16. The entire electron optical system of the apparatus is disposed in a vacuum chamber 18 which can be evacuated and filled with air by devices which are not shown here. The electron beam impinges with its focus on a sample

holder 20 whose properties are described in greater detail hereinafter with reference to Figures 3 through 6. The electron beam can be deflected by means of the deflection unit 14 in two orthogonal directions perpendicularly to the beam direction, independently of each other, so that the electron beam focus can be passed in a scanning raster form over a converter layer (see Figures 3 through 6) of the sample holder 20 and nano light sources 22 contained in the converter layer excited to light up. The light emitted in different directions by the light source 22 is indicated by arrows in Figure 1.

While the electron beam guide means is disposed completely in the vacuum, a sample 24, on the side of the sample holder which is remote from the electron beam, is arranged under normal pressure. The sample is stably fixed on the sample holder 20 at the near-field spacing by an adhesion layer (not shown here). The light produced by non-elastic electron scatter by the nano light source 22 in the converter passes through the sample 24 and is collected in the optical far field by a high-aperture detection optical means 26. The beam path is indicated by boundary lines 28. The image of the captured light signal is produced on a light-sensitive layer of a photodetector 30, converted into an electrical signal corresponding to the intensity thereof, and fed to an image producing and image processing unit (not shown). In the view which is not to scale in Figure 1, the sample 24 and the sample holder 20 are shown on a greatly enlarged scale.

Figure 2 shows a modified arrangement 10′ of the embodiment of Figure 1 for samples which can be exposed to vacuum. The same references denote the same components, in comparison with the foregoing example of Figure 1. In the present embodiment, the sample 24 is arranged in the vacuum chamber 18 and has the electron beam 13 passing therethrough. The sample 24 is fixed on a sample holder 20′. Unlike Figure 1, in this embodiment the sample holder 20′ can be thicker. The sample holder 20′ otherwise differs from the sample holder 20 in

Figure 1 in that its adhesion layer is arranged on the side which is towards the electron source.

The electron beam 13 causes excitation of a nano light source 22 for the emission of light which passes through the sample and the image of which is formed laterally on the detector 20 through a high-aperture objective lens 26'.

Figure 3 shows a first preferred embodiment of a sample holder 20 according to the invention. The electron beam 13 which comes from below in the drawing firstly passes through an electron window 32, then penetrates a carrier layer 20.1 on which a converter layer 20.2 is disposed. In the converter layer 20.2 the electron beam 13 is greatly retarded by non-elastic scatter. The energy transmitted to the converter material excites it to emit light. The region within the converter, in which the electrons are retarded represents the nano light source 22.

The converter layer 20.2 is protected from contamination and oxidation by a protective layer 20.3. Disposed in turn on the protective layer 20.3 is an adhesion layer 20.4 which serves for mounting the sample 24. Only the part of the sample 24 which is in the near field of the light source contributes substantially to the optical signal which is measured in the far field.

In the present embodiment the sample 24 is arranged in a nutrient solution container 34 which contains a nutrient solution indicated by broken-line hatching.

The position of the nano light source 22 and therewith simultaneously the position of the sample at which measurements are taken are altered by movement of the focus of the electron beam 13 in the plane of the converter layer 20.2. The near-field image of the sample is obtained by the representation of the intensity of the optical signal, in dependence on the location of the light source.

Figure 4 shows a sample holder 34, as an alternative embodiment of the invention. The electron beam 13 which comes from below in the drawing passes through a carrier layer 34.1. Arranged in the upper region

of the carrier layer 34.1 in small volumes 34.2 is a converter material 34.3 which is produced by transformation of the carrier material. This involves microporous silicon. The converter material 34.3 is excited to produce intensive light by virtue of electron beam excitation with the electron beam. A protective layer 34.4 protects the microporous silicon 34.3 from contamination. The protective layer is in turn covered by an adhesion layer 34.5 which serves for fixing the sample 24 (not shown here). Otherwise the further structure of the sample holder can be identical to the description with reference to Figure 3.

Figure 5 shows a sample holder 36 with a carrier structure 36.1, as a further embodiment of the invention. Unlike the sample holder 34 shown in Figure 4, provided in the carrier structure 36.1 are conical holes 36.2, in which a converter material 36.3 is crystallized out by evaporation of the solvent of a solution of anthracene or scintillators. Upon electron beam excitation the converter material 36.3 intensively emits light and is used as described with reference to Figure 3 for imaging purposes.

Figure 6 shows a sample holder 38 as an embodiment as an alternative to the structure shown in Figure 3. A particularly thin carrier matrix 38.1 is made possible by virtue of the fact that it is reinforced by support structures 38.2 which are spaced from each other at a relatively large spacing of between 10 and 100 μm. The support structures carry the forces of the pressure difference between the vacuum on the side towards the electron source 12 (Figure 1) and the normal pressure on the side remote from the electron source 12. The particularly thin carrier layer affords the advantage that the electron beam 13 is only slightly scattered. In that way the extent of the nano light source 22 excited to produce light (see Figure 1) is reduced, whereby ultimately the lateral resolution of the near-field microscope is increased.

CLAIMS

1. A sample holder (20; 20′; 34; 36; 38), in particular for a biological sample (24), for use in an apparatus (10; 10′) for near-field optical imaging, comprising a carrier (20.1; 34.1; 36.1; 38.1, 38.2) and a converter (20.2; 34.2, 34.3; 36.3, 36.3; 38.3) which is connected to the carrier and which contains a converter material (34.3, 36.3) and is adapted to emit light of small lateral source extent upon irradiation with an electron beam (13), characterized by an adhesion layer (20.4; 34.5; 36.5; 38.5) which is arranged and designed to fix the sample (24) in the near field of the converter (20.2; 34.2, 34.3; 36.3, 36.3; 38.3).

2. A sample holder as set forth in claim 1 characterized in that the adhesion layer (20.4; 34.5; 36.5; 38.5) is of a thickness of a maximum of 30 nm.

3. A sample holder as set forth in claim 1 or claim 2 characterized in that the thickness of the adhesion layer (20.4; 34.5; 36.5; 38.5) is constant over a portion of its lengthwise and transverse extent.

4. A sample holder as set forth in one of claims 1 through 3 characterized in that the adhesion layer (20.4; 34.5; 36.5; 38.5) includes a lipid or a cellulose derivative.

5. A sample holder as set forth in one of claims 1 through 4 characterized in that the adhesion layer (20.4; 34.5; 36.5; 38.5) is in the form of a molecule mono-layer or molecule bi-layer.

6. A sample holder as set forth in claim 4 or claim 5 characterized in that the adhesion layer (20.4; 34.5; 36.5; 38.5) includes a phospholipid.

7. A sample holder as set forth in claim 4, claim 5 or claim 6 characterized in that the adhesion layer (20.4; 34.5; 36.5; 38.5) contains a carboxylmetoxy cellulose.

8. A sample holder as set forth in one of the preceding claims characterized by a converter material (34.3; 36.3) which upon electron irradiation emits light of a spectral width of more than 50 nm wavelength.

9. A sample holder as set forth in one of the preceding claims characterized in that the converter material (34.3; 36.3) is embedded in small volumes (34.2; 34.3) in the carrier.

10. A sample holder as set forth in claim 9 characterized in that the volumes (34.2; 36.2) are regularly repetitively arranged at a small relative spacing in two directions.

11. A sample holder as set forth in one of the preceding claims characterized in that the converter material (36.3) includes a phosphorus.

12. A sample holder as set forth in one of claims 1 through 11 characterized in that the converter material (36.3) includes anthracene.

13. A sample holder as set forth in one of the preceding claims characterized in that the converter material (34.3) includes transformed material of the carrier (34.1) in small volumes (34.2).

14. A sample holder as set forth in one of the preceding claims characterized in that the converter material (34.3) contains micro- or nano-porous silicon.

15. A sample holder as set forth in one of claims 1 through 8 and 11 through 14 characterized in that the converter material (20.2; 38.3) is homogenously distributed on the carrier (20.1; 38.1).

16. A sample holder as set forth in claim 14 characterized in that the carrier forms the converter.

17. A sample holder as set forth in one of the preceding claims characterized in that the carrier (20.1; 34.1; 36.1; 38.1, 38.2) comprises a semiconductor material, preferably Si, SiO_2, Si_3N_4 or GaAs.

18. A sample holder as set forth in one of the preceding claims characterized in that the carrier (38.1) is reinforced at spacings of between 10 and 100 μm by an additional thickening (38.2).

19. A sample holder as set forth in one of the preceding claims characterized by a window (32) which is transmissive for electrons, on the side of the carrier (20.1) which is remote from the adhesion layer (20.4), the window preferably containing beryllium or silicon.

20. A sample holder as set forth in claim 19 characterized in that the window (32) is designed and arranged to reinforce the carrier (20.1).

21. A sample holder as set forth in one of the preceding claims characterized by a protective layer (20.3; 34.4; 36.4; 38.4) between the converter (20.2; 34.2, 34.3; 36.3, 36.3; 38.3) and the adhesion layer (20.4; 34.5; 36.5; 38.5) which is adapted to protect the converter from contamination by the sample (24).

22. A sample holder as set forth in one of the preceding claims characterized by a nutrient solution container (34) which is releasably

fixed to the sample holder and which is adapted to receive the sample (24) and a nutrient solution.

23. Apparatus (10; 10′) for near-field optical imaging comprising an electron beam source (12) adapted to emit an electron beam (13) and a sample holder, characterized in that the sample holder (20; 20′; 34; 36; 38) is designed in accordance with one of claims 1 through 21.

24. Apparatus as set forth in claim 23 characterized by a vacuum arrangement (18) which is designed in such a way that a converter (20.2; 34.2, 34.3; 36.3, 36.3; 38.3) connected to the sample holder (20; 20′; 34; 36; 38) can be excited in vacuum by the electron beam (13).

25. Apparatus as set forth in one of claims 23 and 24 characterized in that the electron beam (13) can be passed by means of a deflection unit (14) in a scanning raster mode over the plane of the sample holder (20; 20′; 34; 36; 38) in which a converter material (20.2; 34.3; 36.3; 38.3) is arranged.

26. Apparatus as set forth in one of claims 23 through 25 characterized in that the vacuum arrangement (18) is such that a sample (24) to be investigated is disposed on the side of the converter (20) remote from the electron beam source (12) in a normal-pressure atmosphere.

27. Apparatus as set forth in one of claims 23 through 26 characterized in that the electron beam source (12) is adapted to emit an electron beam with an electron energy of between 100 eV and 30 keV.

28. Apparatus as set forth in one of claims 23 through 27 characterized by spectral analysis means (30) which are arranged and

designed to use light emitted by the converter material (20.2; 34.3; 36.3; 38.3) or the sample (24) for spectral analysis of the sample.

29. A production process for a sample holder (20; 20'; 34; 36; 38) for use in an apparatus (10; 10') for near-field optical imaging, in which a carrier (20.1; 34.1; 36.1; 38.1,38.2) is provided with a converter (20.2; 34.2, 34.3; 36.3, 36.3; 38.3) and in which the carrier is then coated with an adhesion layer (20.4; 34.5; 36.5; 38.5), wherein the adhesion layer (20.4; 34.5; 36.5; 38.5) is applied in the form of mono- or bi-layers by attraction or by bursting vesicles.

30. A process as set forth in claim 29 characterized by a step of coating the carrier (20.1; 34.1; 36.1; 38.1, 38.2) with a protective layer (20.3; 34.4; 36.4; 38.4), in which a polyacryl is firstly applied as a monomer and then cross-linked by UV-irradiation to provide a two-dimensional mono-layer.

Abstract

The invention concerns a sample holder (20), in particular for a biological sample (24), for use in an apparatus for near-field optical imaging, comprising a carrier (20.1) and a converter (20.2) which is connected to the carrier and which contains a converter material and is adapted to emit light of small lateral source extent upon irradiation with an electron beam (13). In accordance with the invention there is provided an adhesion layer (20.4) which is arranged and adapted to fix the sample (24) in the near field of the converter (20.2).

Figure 3

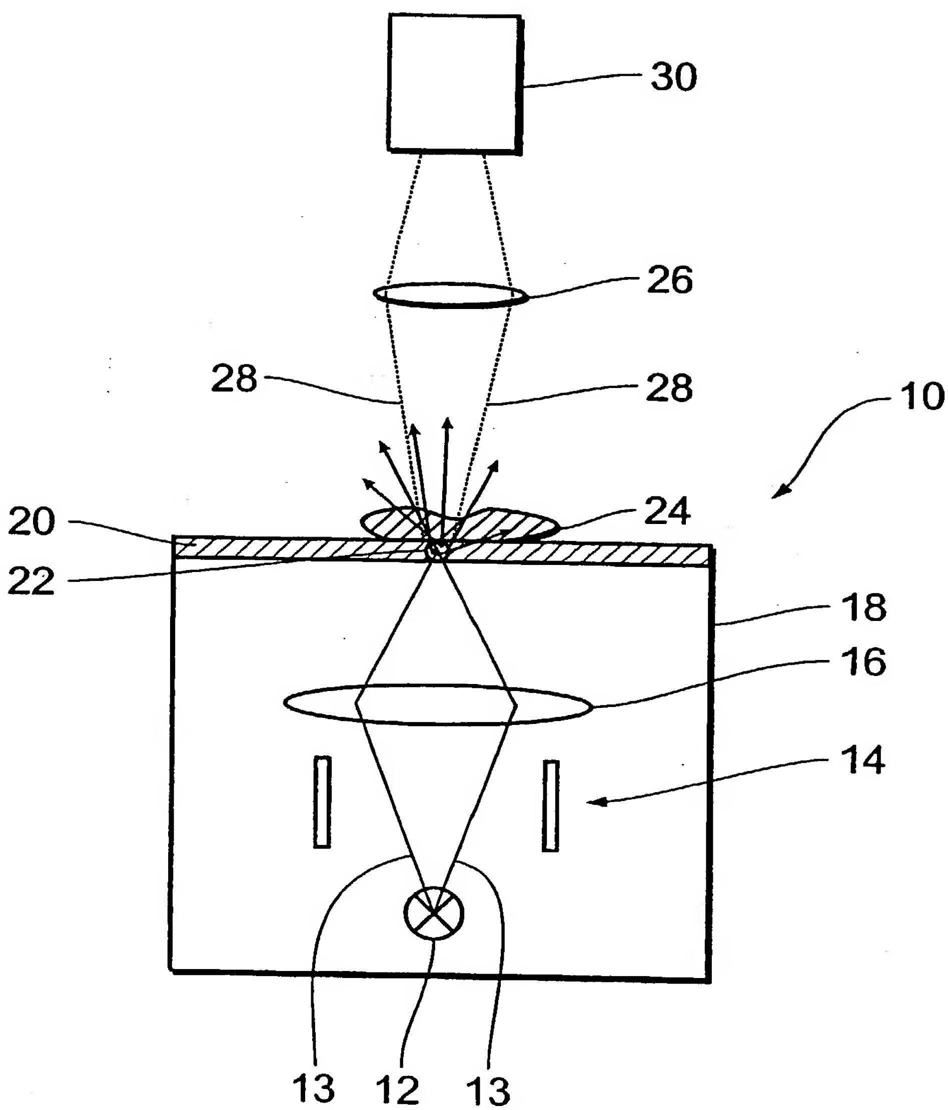

Fig. 1

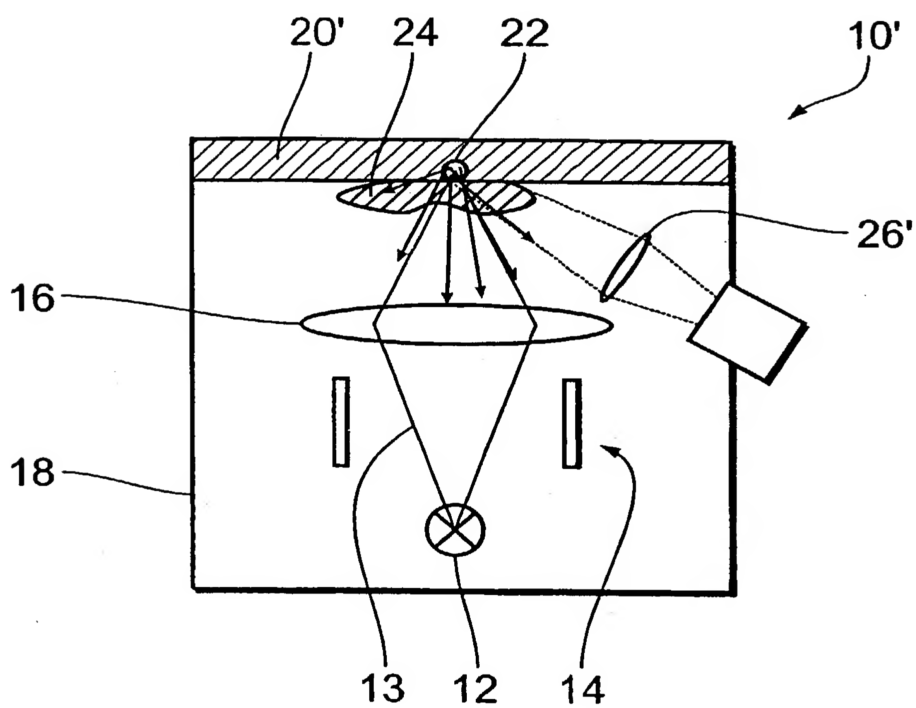

Fig. 2

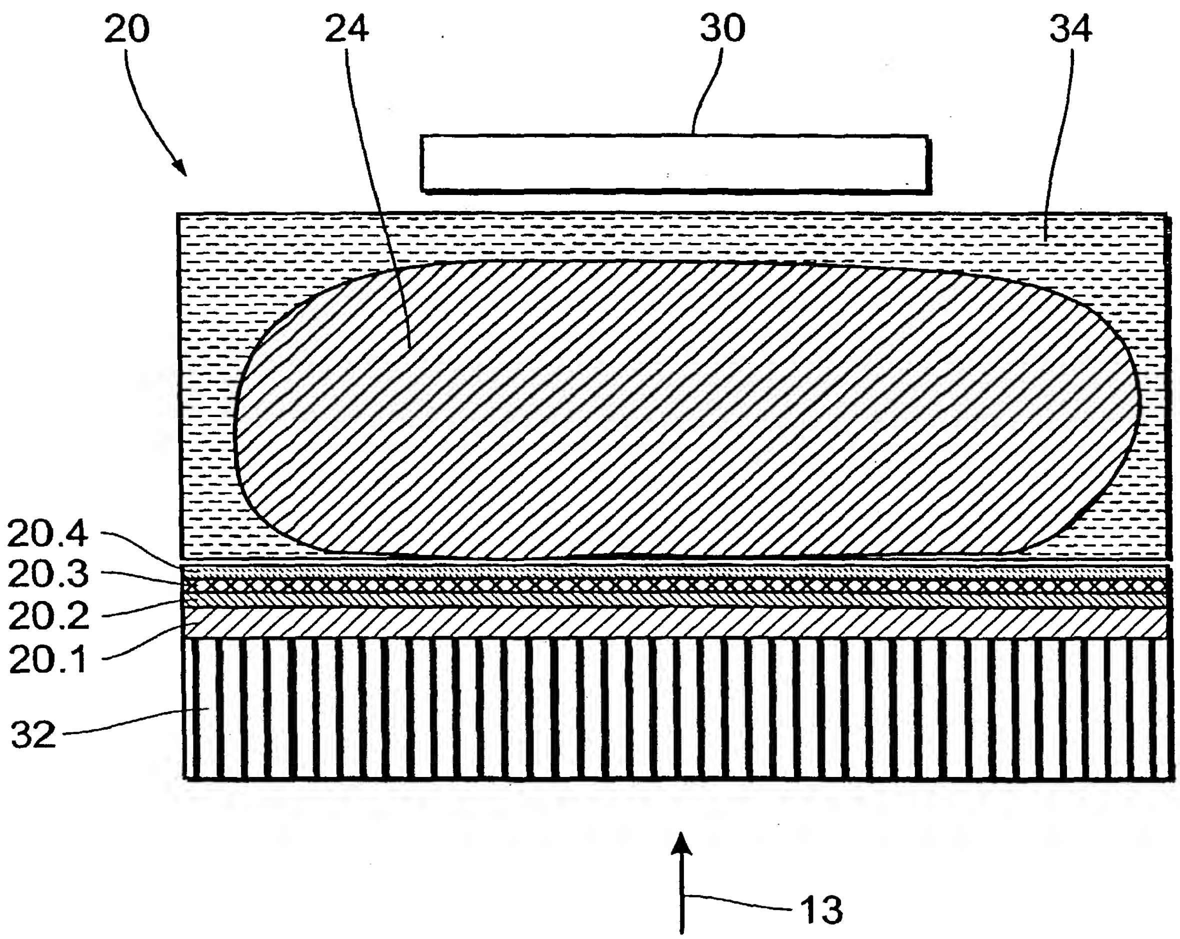

Fig. 3

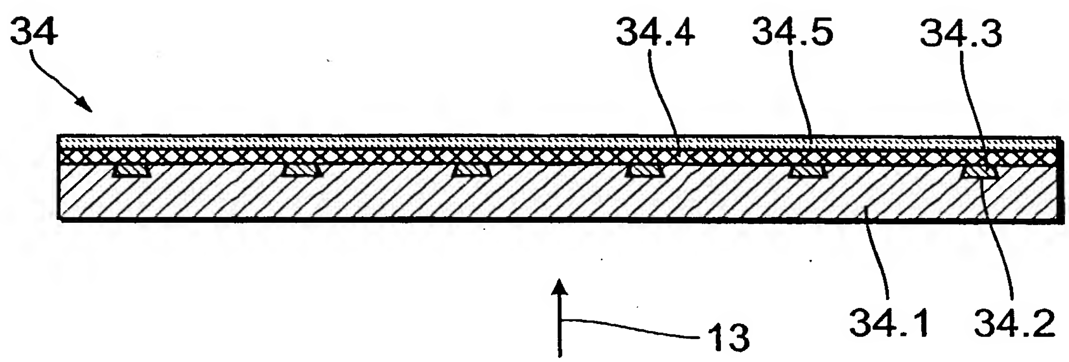

Fig. 4

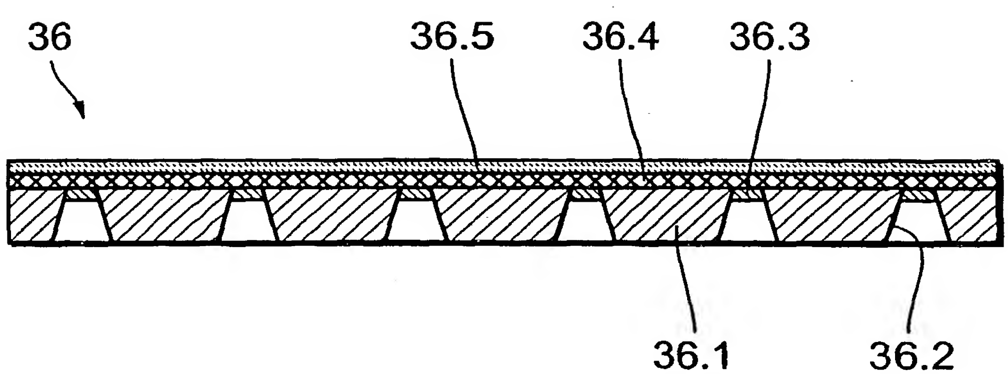

Fig. 5

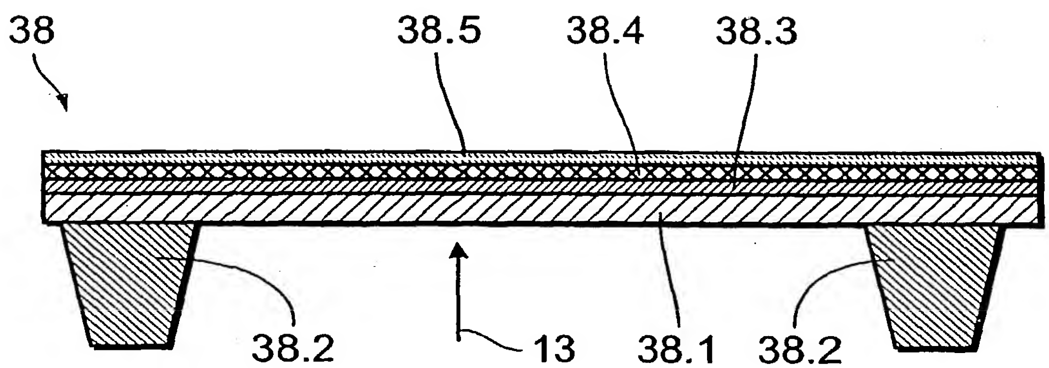

Fig. 6

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION, OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is for a national stage of PCT application.

INVENTORSHIP IDENTIFICATION

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am an original, first and joint inventor of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

NEAR FIELD OPTICAL EXAMINATION DEVICE

SPECIFICATION IDENTIFICATION

The specification was described and claimed in PCT International Application No. EP00/06088 filed on June 29, 2000 .

ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, Section 1.56, and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent .

PRIORITY CLAIM (35 U.S.C. Section 119(a)-(d))

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

Such applications have been filed as follows.

PRIOR FOREIGN APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. SECTION 119(a)-(d)

COUNTRY	APPLICATION NUMBER	DATE OF FILING DAY, MONTH, YEAR	PRIORITY CLAIMED UNDER 35 U.S.C. SECTION 119
Germany	199 29 875.0	29 June 1999	yes

POWER OF ATTORNEY

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Scott M. Oldham	32712
Mark A. Watkins	33813

9

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330-864-5550

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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